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MOLECULES IN FOCUS

PKR—A Protein Kinase Regulated by Double-stranded RNA

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The RNA-regulated protein kinase, (PKR) is an interferon-inducible enzyme of widespread occurrence in eukaryotic organisms. This serine/threonine-specific protein kinase is activated by double-stranded RNA by a mechanism involving autophosphorylation. Once activated, the enzyme phosphorylates the α subunit of protein synthesis initiation factor eIF2, thereby inhibiting translation. Recent evidence suggests that there may be additional substrates, and that signal transduction and gene transcription pathways also may be regulated by the protein kinase. As well as being important in mediating the antiviral effects of interferons, PKR is implicated in regulating cell proliferation in uninfected cells and may have a tumour suppressor function under normal conditions. Studies using cell lines expressing inactive mutants of PKR and mice with homozygous disruptions of the PKR gene are leading to greater insights into the biological significance of this enzyme. © 1997 Elsevier Science Ltd

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INTRODUCTION

The interferons (IFNs) originally were identified as agents produced by virus-infected cells, which can induce an antiviral state when uninfected cells subsequently are exposed to them. It later became apparent that these cytokine-like molecules have a wide range of additional effects on cells, including inhibition of proliferation, induction of differentiation, and modulation of immunological pathways (e.g. enhancement of histocompatibility antigen expression) (Pestka *et al.*, 1987). Cells treated with IFNs exhibit increased sensitivity of protein synthesis to inhibition by double-stranded RNA (dsRNA), and this constitutes one of the major ways by which viral replication is impaired. The main cause of the increased sensitivity to dsRNA is the transcriptional induction of the protein kinase PKR; this enzyme, along with the family of 2′5′ oligoadenylate synthetases, is activated by dsRNA produced during the course of viral replication and leads to an inhibition of translation in virus-infected cells. Since the cDNA for PKR from human cells was cloned and sequenced in 1990 (Meurs *et al.*, 1990), there has been an explosion of interest in the properties and biological roles of the protein kinase. The impetus for much of this work has come from the fact that PKR may not only be an inhibitor of translation with antiviral properties, but also may be a growth inhibitory protein that functions as a tumour suppressor (Meurs *et al.*, 1993). Recent studies also suggest that protein synthesis is not the only pathway that is a target for regulation by PKR, and that the protein kinase may control certain signal transduction and transcriptional events in uninfected cells. This article will describe briefly some of these aspects. For other recent reviews

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on PKR, see Proud (1995), Williams (1995) and Clemens (1996).

STRUCTURE

Sequences of cDNAs encoding human, mouse and rat PKR have now been described, together with the structure of the entire mouse gene (Tanaka and Samuel, 1994). The latter comprises 16 exons and is transcribed from a promoter that contains a large number of potential regulatory elements. The structure of the protein itself is of considerable interest, since two discrete modules can be discerned. One of these contains the 11 structural motifs characteristic of protein kinases in general, present in the C-terminal half of the protein. In addition, near the N-terminus, there are two partially repeated dsRNA-binding motifs, which are necessary for the interaction of the enzyme with its dsRNA activators (Clemens, 1996) (see Fig. 1).

SYNTHESIS AND DEGRADATION

The PKR gene is expressed in a wide range of cell types and appears to be active constitutively at a low level under most physiological conditions. It is induced strongly at the transcriptional level within a few hours of IFN treatment, and intracellular concentrations of the protein rise by a factor of from five- to 10-fold under these circumstances. Because of the complexity of the promoter, it is likely that expression of the PKR gene is subject to control by a wide variety of influences in addition to the IFNs, although relatively little has been done to investigate the transcriptional regulation of this protein. There is also some evidence for translational control of PKR synthesis, and it has been suggested that feedback occurs at this level because of the ability of PKR to impair its own translation.

Studies on the subcellular distribution of PKR have shown that, although approximately 80% of the protein is cytoplasmic (mostly associated with ribosomes), a significant fraction occurs in the nucleolus (Jeffrey *et al.*, 1995). The significance of the latter fraction is not known. The enzyme is phosphorylated extensively at multiple sites, not all of which have yet been mapped, but the nucleolar form is substantially less phosphorylated than the cytoplasmic form of the kinase. PKR is a reasonably stable protein *in vivo*, and so far there is no evidence for regulation of its concentration through changes in degradation rate.

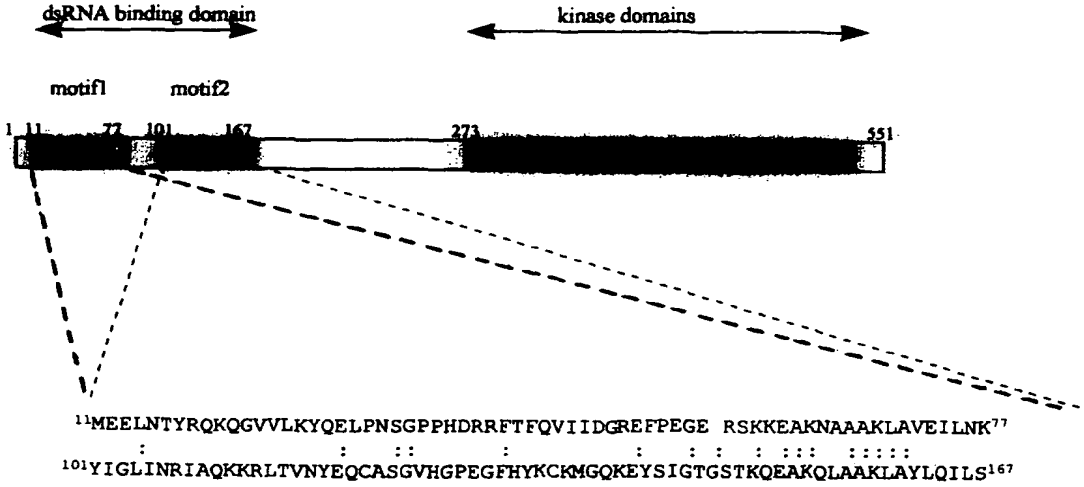

Fig. 1. Domain structure of the protein kinase PKR. The molecule consists of 551 amino acids and can be divided into two principal regions. The 11 domains characteristic of serine/threonine protein kinases are in the C-terminal half (amino acids 273–526, shown here in blue) (Meurs *et al.*, 1990). In addition, two partially homologous dsRNA binding domains occur near the N-terminus (amino acids 11–77 and 101–167, shown here in red). The amino acid sequences of the dsRNA binding domains are presented in the lower part of the figure, with sequence identities between them indicated by a colon. Residues that have been shown by site-directed mutagenesis to be critical for dsRNA binding are indicated in red. The structure and sequence information in this figure refer to human PKR, but the mouse and rat enzymes possess similar features.

BIOLOGICAL FUNCTION

There are two important aspects to consider in understanding the function of PKR. One is the regulation of its conversion from a latent to an active protein kinase; the other is its ability to phosphorylate its substrates and the consequences of this for its biological effects on the cell.

Current evidence suggests that there are two requirements for the activation of PKR. One is the binding of dsRNA to one or both motifs near the N-terminus (Fig. 1); the other is the necessity for dimerization of the protein. The latter may come about as a consequence of the association of two (or more) molecules of PKR with a single dsRNA molecule; however, there is also strong evidence that direct protein–protein interactions also can cause dimerization, independently of the RNA ligand. To add to the confusion, the same N-terminal regions of the protein that bind dsRNA are required also for this protein–protein association. It will he important to establish the detailed nature of the dimerization phenomenon, because it has implications for the mode of action of dominant negative mutants of PKR, which have been shown to cause tumorigenic transformation when stably expressed in NIH 3T3 cells (Meurs *et al.*, 1993). Currently, there is evidence for two models of how these mutants act to inhibit wild-type PKR activity. One idea is that the mutants sequester limiting amounts of cellular dsRNA activators, whereas an alternative suggestion is that there is direct heterodimerization between mutant and wild-type proteins, which blocks the activation of the wild-type PKR.

We should bear in mind also the possibility that PKR can be activated *in vivo* by a dsRNA-independent mechanism; e.g. treatment of uninfected cells with the Ca^{2+} ionophore A23187 activates the kinase and causes inhibition of protein synthesis, although the mechanism has not been established. A cellular protein, p58, has been shown to be an endogenous inhibitor of PKR and may serve to limit the activation of the protein kinase under many physiological conditions (Polyak *et al.*, 1996).

By far the best characterized *in vivo* substrate for the protein kinase activity of PKR is the smallest (α) subunit of polypeptide chain initiation factor eIF2. Phosphorylation of this protein on Ser^{51} inhibits protein synthesis by blocking the guanine nucleotide exchange reaction required to recycle eIF2 from an inactive GDP-associated form to an active GTP-associated state. This reaction is catalysed by the factor eIF2B, which effectively becomes sequestered by binding tightly to phosphorylated [eIF2.GDP]. It is likely that other PKR substrates also exist in the cell, and *in vitro* evidence has been presented for the phosphorylation by PKR of the transcriptional regulatory protein IκB (the inhibitor of NFκB), as well as the HIV-encoded Tat86 protein. However, it is not clear whether these proteins are substrates for PKR *in vivo* and, although there is good evidence for a PKR requirement for the regulation of NFκB-dependent gene expression by dsRNA (Maran *et al.*, 1994), the process may be an indirect one.

The biological effects of PKR are manifold. The protein kinase is an important mediator of many of the antiviral effects of the IFNs and, together with the IFN-inducible 2′5′ oligoadenylate synthetases, contributes to the shut-off of protein synthesis following infection (Katze, 1995). In many cases, viral dsRNAs are probably the activators of this process. However, it is clear that PKR can exert effects in uninfected cells also and that it can be a potent growth inhibitory protein when activated. It is not clear which function may have arisen first during the course of evolution, i.e. whether the antiviral activity of PKR represents a specialized aspect of a more general inhibitory effect towards translation, or whether the protein kinase initially evolved as a cellular defence mechanism, which then acquired more general functions occurring in the absence of infecting viruses.

Observations that PKR is growth inhibitory when expressed in both mammalian cells and yeast, together with the fact that inactive mutant forms of the enzyme can transform 3T3 cells, have led to the suggestion that PKR is a tumour suppressor protein. How it may exert this effect is not yet known; one possibility is that, when protein synthesis is down-regulated as a result of the partial phosphorylation of eIF2α by PKR, the rate of synthesis of growth-stimulatory proteins (e.g. growth factors or proto-oncogene products) is preferentially impaired. Such proteins are often translated from 'inefficient' mRNAs, which would be especially sensitive to sub-optimal conditions of protein synthesis. This model is supported by the observation that mutation of Ser^{51} in eIF2α to a non-phosphorylatable alanine also causes tumorigenic transformation of 3T3

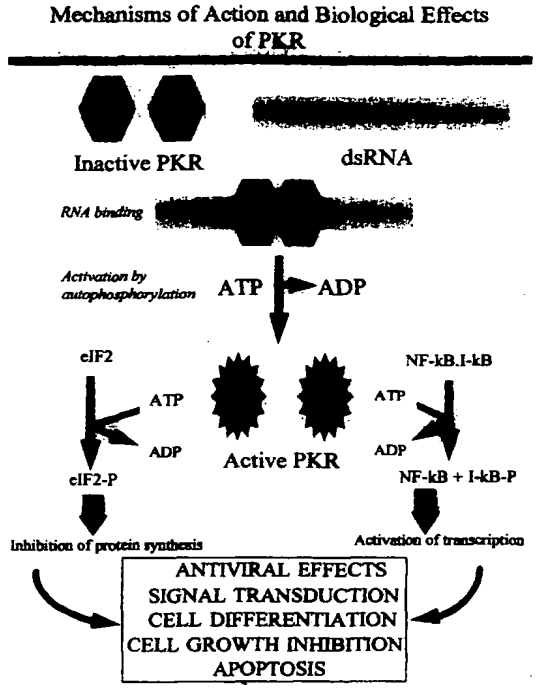

Fig. 2. Mechanisms of action and biological effects of PKR. Two molecules of monomeric PKR dimerize and bind to an activating dsRNA molecule. The protein kinase then becomes activated by autophosphorylation and probably also undergoes a conformational change. This renders it independent of dsRNA and allows it to phosphorylate its protein substrates. Many of the biological effects of PKR (particularly those relating to changes in cellular protein synthesis) probably consequences of the phosphorylation of the α subunit of polypeptide chain initiation factor eIF2; other actions of PKR may involve activation of the NFκB family of transcription factors (via direct or indirect phosphorylation of IκB inhibitors), or possibly other transcription factors. The protein kinase is known to be functional as both an antiviral protein and an inhibitor of cell proliferation (and potential tumour suppressor gene product). Also, it may have roles in the mediation of signal transduction events in response to growth factors such as PDGF and IL-3 (reviewed in Williams, 1995), in the regulation of apoptosis (Lee and Esteban, 1994), and in the transcriptional control of gene expression.

cells (Donze *et al.*, 1995). However, the relative rates of translation of different mRNAs have not yet been examined in these cells. An alternative mechanism for the growth-regulatory effects of PKR is that the kinase has specific transcriptional effects (perhaps through the regulation of NFκB), which alter the state of growth or differentiation of the cells in which the enzyme is active. These potential mechanisms are summarized in Fig. 2.

The biological roles and mechanisms of action of PKR have begun to be addressed by the generation of homozygous PKR knockout mice (Yang *et al.*, 1995). However, in spite of evidence of deficient signalling and impairment of the activation of NFκB in response to dsRNA in these animals, the mice develop normally and show no increased rates of spontaneous tumour formation. It will be of great interest to examine the regulation of the phosphorylation of eIF2α under various conditions in cells derived from these animals, to see whether this process is impaired.

POTENTIAL MEDICAL APPLICATIONS

In view of its potent antiviral and possible anti-tumour properties, PKR obviously has at least a theoretical potential as an agent to attack a number of serious medical problems. Targeted expression of the enzyme in selected cell types, or the delivery of specific activators of PKR to infected or malignant tissues, might prove beneficial in a more specific way than can be achieved with the blunt-edged weapon of systemic IFN therapy. However, before such goals can be achieved, clearly we need to learn much more about the regulation and physiological roles of this protein kinase in normal cells.

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